Journal of Forensic and Legal Medicine 17 (2010) 247–253

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Original Communication

Mitochondrial DNA sequence analysis of a native Bolivian population

H. Afonso Costa Msc, Intern [a,*], M. Carvalho Msc, Forensic Expert [a], V. Lopes Bsc, Forensic Expert [a], F. Balsa Msc, Intern [a], A.M. Bento Msc, Intern [a], A. Serra Bsc, Forensic Expert [a], L. Andrade Msc, Forensic Expert [a], M.J. Anjos Bsc, Director [a], M.C. Vide Bsc, Former Director [a], S. Pantoja Phd, Professor [b], D.N. Vieira Phd, President [c,d], F. Corte-Real Phd, Director [c,d]

[a] *Forensic Genetics Service, Centre Branch, National Institute of Legal Medicine, Largo da Sé Nova, 3000-213 Coimbra, Portugal*
[b] *University of La Paz, Bolivia*
[c] *National Institute of Legal Medicine, Portugal*
[d] *Faculty of Medicine, University of Coimbra, Portugal*

A R T I C L E I N F O

Article history:
Received 10 July 2009
Received in revised form
4 December 2009
Accepted 13 February 2010
Available online 31 March 2010

Keywords:
Mitochondrial DNA typing
Hypervariable region I
Hypervariable region II
Haplogroup
Bolivia

A B S T R A C T

Mitochondrial DNA analysis is very useful for the interpretation of the history of human migration and to estimate the frequency of a haplotype in the forensic context. From a human settlement perspective, La Paz area is greatly interesting since the first planned city of the region is located there. Samples from 110 individuals from La Paz were studied analysing the polymorphisms in the D-loop, hypervariable region I (HVI) and hypervariable region II (HVII) in order to verify the genetic diversity. The aim of this study was to start the creation of a population database in order to obtain the genetic interpopulation variability and classify haplotypes into characteristic haplogroups of South America. A total of 97 different haplotypes were identified, 90 being unique, expressed by 122 polymorphic nucleotide positions. Nucleotide and sequence diversity were estimated to be 0.015 ± 0.0075 and 0.996, respectively. Haplogroup distribution in the samples was 57.27% B4, 19.09% C1, 10.00% A2, 3.64% D1, 2.73% D4h3, 1.82% H, and 0.91% for each of the haplogroups A4, B4c1a, CZ, D4J, M7a and M8/N9b. The rate of length heteroplasmy was 36.36% in HVI and 52.73% in HVII. Phylogenetic analysis reveals proximity to the Korean, Chilean aboriginal, Japanese and Australian populations. The estimated genetic variability of the studied population was high, suggesting an early settlement.

1. Introduction

Bolivia is a South American country in the heart of the Andes. The history of the territory follows the cultures of the first empire within the domains of the first planned city of the region, Tiwanaku, sited in the South coast of the Lake Titicaca. The last continent to be populated by humans was the Americas and several questions remain open regarding this event. Due to its antiquity, the Andean region can reveal some of the human migration and colonization history of the New World since most of the Bolivian population descends from Aymaras and Quechuas, two cultural groups from the Tiwanaku and Inka Empire, which persisted labouring the fields of the Andean highlands around Lake Titicaca until the present days.[1]

Mitochondrial DNA (mtDNA) is an excellent tool for population studies and forensic genetics due to its high copy number per cell, maternal inheritance,[2] high mutation rate of evolution that provide highly polymorphism to the sequences and absence of recombination.[3] The discrimination power of mtDNA is due to the polymorphic nature of the hypervariable regions (HVI, HVII, and HVIII) which are located in the control region. The mitochondrial genome has become the most widely used genetic marker in human evolution studies. Sequences with shared mutations that tend to reveal a regional specificity define haplogroups. These haplogroups are important to clarify the history and demographic past of a population since they can reflect a phylogenetic relationship between populations.[4] The first mtDNA haplogroups discovered in Native Americans were determined by Torroni et al., 1993.[5] Posterior studies of mtDNA variation concluded that Native American populations have five distinct major haplogroups, all of Asian origin (A, B, C, D and X).[5,6] For forensic purposes, the discrimination power between individuals based on mtDNA is lower than traditional nuclear DNA markers because of its maternal inheritance; however, mtDNA analysis is particularly useful when there are degraded or low level DNA samples. To solve cases of missing persons and to

* Corresponding author. Tel.: +351 239 854 230; fax: +351 239 820 549.
E-mail address: afonsocostah@gmail.com (H. Afonso Costa).

1752-928X/$ – see front matter
doi:10.1016/j.jflm.2010.02.011

Table 1
HVI and HVII mtDNA sequences of 110 individuals from La Paz. N – number of occurrence of the haplotype; Est. Hg – "estimated haplogroup".

	HVI	HVII	N	Est. Hg
LPAZ001	16183C; 16189C; 16217C[a]	73G; 207A; 263G; 315.1C	1	B4
LPAZ002	16223T; 16292T; 16325C; 16362C	73G; 263G; 315.1C	1	D1
LPAZ003	16183C; 16189C; 16217C[a]	73G; 103A; 263G; 315.1C	1	B4
LPAZ004	16093C; 16183C; 16189C; 16217C[a]	73G; 263G; 309.1C; 315.1C	1	B4
LPAZ005	16183C; 16188T; 16189C; 16217C	73G; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ006	16183C; 16188T; 16189C; 16217C	73G; 185A; 263G; 309.1C; 315.1C	1	B4
LPAZ007	16183C; 16188T; 16189C; 16217C	73G; 241G; 263G; 309.1C; 315.1C[a]	1	B4
LPAZ008	16183C; 16189C; 16217C[a]	73G; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ009	16223T; 16325C; 16362C	73G; 143A; 263G; 309.1C; 315.1C	1	D1
LPAZ010	16183C; 16189C; 16217C[a]	73G; 146C; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ011	16183C; 16189C; 16217C; 16270T; 16278T[a]	73G; 263G; 309.1C; 315.1C[a]	1	B4
LPAZ012	16183C; 16188T; 16189C; 16217C; 16362C	73G; 152C; 186T; 263G; 315.1C	1	B4
LPAZ013	16182C; 16183C; 16189C; 16217C[a]	73G; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ014	16183C; 16189C; 16217C[a]	73G; 143A; 146C; 215G; 263G; 315.1C	1	B4
LPAZ015	16168T; 16183C; 16189C; 16217C; 16270T; 16278T[a]	73G; 263G; 315.1C	1	B4
LPAZ016	16223T; 16241G; 16304C; 16325C; 16362C	73G; 263G; 309.1C; 315.1C	1	D1
LPAZ017	16168T; 16182C; 16183C; 16189C; 16217C; 16218T[a]	73G; 263G; 315.1C	1	B4
LPAZ018	16183C; 16188T; 16189C; 16217C	73G; 151T; 186T; 204C; 263G; 315.1C	1	B4
LPAZ019	16182C; 16183C; 16189C; 16217C; 16362C[a]	73G; 263G; 309d; 315.1C;	1	B4
LPAZ020	16183C; 16189C; 16217C; 16293C[a]	73G; 204C; 207A; 263G; 315.1C[a]	1	B4
LPAZ021	16183C; 16189C; 16217C[a]	73G; 151T; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ022	16183C; 16188T; 16189C; 16217C; 16354T	73G; 186T; 263G; 309.1C; 315.1C	1	B4
LPAZ023	16183C; 16189C; 16217C[a]	73G; 143A; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ024	16183C; 16188T; 16189C; 16217C; 16266T	73G; 186T; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ025	16183C; 16188T; 16189C; 16217C; 16354T; 16362C	73G; 186T; 263G; 309.1C; 315.1C[a]	1	B4
LPAZ026	16183C; 16189C; 16217C[a]	73G; 103A; 143A; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ027	16183C; 16189C; 16217C; 16261T; 16319A[a]	73G; 75A; 263G; 315.1C; 340T[a]	1	B4
LPAZ028	16129A; 16183C; 16189C; 16217C[a]	73G; 146C; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ029	16183C; 16189C; 16217C; 16289G[a]	73G; 143A; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ030	16183C; 16189C; 16217C; 16356C; 16362C[a]	73G; 204C; 263G; 309.1C; 315.1C[a]	1	B4
LPAZ031	16183C; 16189C; 16217C[a]	73G; 207A; 257G; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ032	16183C; 16189C; 16217C; 16359C[a]	73G; 143A; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ033	16183C; 16188T; 16189C; 16217C; 16354T	73G; 146C; 186T; 263G; 309.1C; 315.1C	1	B4
LPAZ034	16223T; 16255A; 16301T; 16342C; 16362C	73G; 152C; 263G; 309.1C; 315.1C[a]	1	D4h3
LPAZ035	16183C; 16189C; 16217C; 16360T[a]	73G; 143A; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ036	16183C; 16189C; 16217C; 16274A; 16319A; 16362C[a]	73G; 263G; 309.1C; 315.1C[a]	1	B4
LPAZ037	16223T; 16266T; 16298C; 16325C; 16327T	73G; 249d; 263G; 290d; 291d; 315.1C	1	C1
LPAZ038	16105K; 16168T; 16183C; 16189C; 16217C; 16301A[a]	73G; 204C; 263G; 309.1C; 315.1C[a]	1	B4
LPAZ039	16183C; 16189C; 16217C[a]	73G; 143A; 146C; 215G; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ040	16183C; 16188T; 16189C; 16217C; 16304C; 16362C	73G; 152C; 186T; 263G; 309.1C; 315.1C	1	B4
LPAZ041	16066G; 16183d; 16186T; 16189C; 16217C	73G; 143A; 210G; 263G; 309.1C; 315.1C	1	H
LPAZ042	16114G; 16129A; 16222T; 16223T; 16325C; 16362C	73G; 143A; 263G; 309.1C; 315.1C	1	D1
LPAZ043	16183C; 16189C; 16217C; 16289G[a]	73G; 143A; 146C; 215G; 263G; 309d; 315.1C	1	B4
LPAZ044	16051G; 16182C; 16183C; 16189C; 16217C[a]	73G; 152C; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ045	16183C; 16189C; 16217C; 16301A[a]	73G; 146C; 197G; 215G; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ046	16192T; 16223T; 16298C; 16325C; 16327T	73G; 249d; 263G; 290d; 291d; 309.1C; 315.1C	1	C1
LPAZ047	16129A; 16223T; 16298C; 16325C; 16327T	73G; 249d; 263G; 290d; 291d; 309.1C; 315.1C	1	C1
LPAZ048	16111T; 16223T; 16290T; 16319A; 16362C	73G; 146C; 153G; 263G; 309.1C; 309.2C; 315.1C[a]	1	A2
LPAZ049	16183C; 16189C; 16217C; 16319A; 16360T[a]	73G; 146C; 150T; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ050	16182C; 16183C; 16189C; 16217C; 16293G; 16311C[a]	73G; 143A; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4c1a
LPAZ051	16183C; 16189C; 16217C; 16362C[a]	73G; 103A; 146C; 151T; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ052	16223T; 16298C; 16325C; 16327T	73G; 79T; 249d; 263G; 290d; 291d; 309.1C; 315.1C[a]	1	C1
LPAZ053	16176T; 16183C; 16189C; 16217C; 16240G; 16289G; 16362C[a]	73G; 204C; 263G; 309.1C; 315.1C[a]	1	B4
LPAZ054	16092C; 16145A; 16223T; 16298C; 16325C	73G; 185A; 249d; 263G; 290d; 291d; 315.1C	1	CZ
LPAZ055	16051G; 16129A; 16182C; 16183C; 16189C; 16217C; 16234A[a]	73G; 152C; 263G; 309.1C; 315.1C[a]	1	B4
LPAZ056	16092C; 16183C; 16189C; 16217C; 16362C[a]	73G; 143A; 146C; 215G; 263G; 309.1C; 315.1C[a]	1	B4
LPAZ057	16182C; 16183C; 16189C; 16217C; 16362C[a]	73G; 146C; 215G; 263G; 309.1C; 309.2C; 315.1C[a]	1	B4
LPAZ058	16223T; 16293G; 16325C; 16362C	97A; 106d; 107d; 108d; 109d; 110d; 111d; 263G; 315.1C	1	D4j
LPAZ059	16223T; 16290T; 16319A; 16362C	73G; 146C; 153G; 182T; 194T; 195C; 235G; 263G; 315.1C	1	A4
LPAZ060	16223T; 16298C; 16311C; 16325C; 16327T	73G; 150T; 249d; 263G; 290d; 291d; 309.1C; 315.1C	1	C1
LPAZ061	16223T; 16298C; 16325C; 16327T	73G; 162Y; 249d; 263G; 290d; 291d; 309.1C; 309.2C; 315.1C[a]	1	C1
LPAZ062	16172C; 16223T; 16256T; 16298C; 16325C; 16327T	73G; 249d; 263G; 290d; 291d; 309.1C; 315.1C	1	C1
LPAZ063	16111T; 16217C; 16223T; 16290T; 16319A; 16343T; 16362C	73G; 146C; 235G; 260A; 263G; 315.1C	1	A2
LPAZ064	16223T; 16298C; 16325C; 16327T; 16345T	73G; 146C; 249d; 263G; 290d; 291d; 309.1C; 315.1C	1	C1
LPAZ065	16223T; 16298C; 16325C; 16327T; 16381C	73G; 152C; 249d; 263G; 290d; 291d; 309.1C; 315.1C[a]	1	C1
LPAZ066	16037G; 16223T; 16298C; 16325C; 16327T	73G; 222T; 249d; 263G; 290d; 291d; 309.1C; 315.1C	1	C1
LPAZ067	16183C; 16189C; 16217C; 16270T; 16278T[a]	73G; 94A; 152C; 183G; 204C; 263G; 309.1C; 315.1C[a]	1	B4
LPAZ068	16093C; 16183C; 16189C; 16223T; 16298C; 16325C; 16327T; 16381C[a]	73G; 249d; 263G; 290d; 291d; 315.1C	1	C1
LPAZ069	16111T; 16217C; 16223T; 16290T; 16319A; 16343T; 16362C	73G; 146C; 153G; 235G; 260A; 263G; 315.1C	1	A2
LPAZ070	16183C; 16188T; 16189C; 16217C	73G; 106d; 107d; 108d; 109d; 110d; 111d; 186T; 263G; 309.1C; 315.1C	1	B4
LPAZ071	16092C; 16111T; 16223T; 16290T; 16319A; 16362C	73G; 146C; 153G; 222T; 235G; 263G; 309.1C; 315.1C	1	A2
LPAZ072	16140C; 16223T; 16298C; 16311C; 16325C; 16327T	73G; 249d; 263G; 290d; 291d; 309.1C; 309.2C; 315.1C[a]	1	C1

Table 1 (*continued*)

	HVI	HVII	N	Est. Hg
LPAZ073	16223T; 16298C; 16325C; 16327T	73G; 146C; 195C; 249d; 263G; 290d; 291d; 309.1C; 315.1C; 340T	1	C1
LPAZ074	16223T; 16298C; 16325C; 16327T	73G; 125C; 127C; 249d; 263G; 290d; 291d; 309.1C; 309.2C; 315.1C[a]	1	C1
LPAZ075	16145A; 16223T; 16270T; 16298C; 16325C; 16327T	73G; 214G; 249d; 263G; 290d; 291d; 309.1C; 315.1C[a]	1	C1
LPAZ076	16111T; 16136C; 16223T; 16268A; 16290T; 16319A; 16362C	73G; 146C; 153G; 185A; 235G; 263G; 309.1C; 315.1C[a]	1	A2
LPAZ077	16111T; 16217C; 16223T; 16290T; 16319A; 16343T; 16362C	73G; 146C; 152C; 153G; 235G; 260A; 263G; 315.1C	1	A2
LPAZ078	16111T; 16183G; 16223T; 16298C; 16325C; 16327T; 16368C	73G; 152C; 249d; 263G; 290d; 291d; 309.1C; 315.1C[a]	1	C1
LPAZ079	16075C; 16223T; 16241G; 16260T; 16294T; 16301T; 16342C; 16362C	73G; 94A; 152C; 195C; 263G; 309.1C; 315.1C	1	D4h3
LPAZ080	16181G; 16189C; 16223T; 16298C; 16325C; 16327T; 16344T[a]	73G; 185A; 249d; 263G; 290d; 291d; 309.1C; 315.1C[a]	1	C1
LPAZ081	16111T; 16217C; 16223T; 16290T; 16319A; 16362C	73G; 146C; 153G; 212C; 215G; 235G; 263G; 309.1C; 309.2C; 315.1C[a]	1	A2
LPAZ082	16051G; 16213A; 16223T; 16247G; 16298C; 16325C; 16327T	73G; 146C; 195C; 249d; 263G; 290d; 291d; 309.1C; 315.1C	1	C1
LPAZ083	16075C; 16223T; 16241G; 16260T; 16294T; 16301T; 16342C; 16362C	73G; 94A; 125C; 127C; 152C; 195C; 263G; 309.1C; 315.1C	1	D4h3
LPAZ084	16041G; 16172C; 16173T; 16192T; 16223T; 16266T; 16298C; 16325C; 16327T; 16346A	73G; 204C; 249d; 263G; 290d; 291d; 315.1C[a]	1	C1
LPAZ085	16111T; 16183C; 16189C; 16217C; 16223T; 16290T; 16319A; 16343T; 16362C[a]	73G; 146C; 153G; 235G; 260A; 263G; 309.1C; 309.2C; 315.1C[a]	1	A2
LPAZ086	16189C; 16192T; 16223T; 16266T; 16298C; 16316G; 16325C; 16327T	73G; 143A; 150T; 249d; 263G; 290d; 291d; 309.1C; 315.1C; 325T; 338T	1	C1
LPAZ087	16189C; 16192T; 16223T; 16266T; 16298C; 16316G; 16325C; 16327T	73G; 143A; 150T; 201G; 249d; 263G; 290d; 291d; 309.1C; 315.1C; 325T	1	C1
LPAZ088	16183C; 16188T; 16189C; 16217C	73G; 186T; 263G; 315.1C	2	B4
LPAZ089	16183C; 16188T; 16189C; 16217C	73G; 186T; 263G; 309.1C; 315.1C	2	B4
LPAZ090	16111T; 16223T; 16290T; 16319A; 16362C	73G; 146C; 153G; 235G; 263G; 309.1C; 315.1C[a]	3	A2
LPAZ091	16183C; 16188T; 16189C; 16217C	73G; 263G; 309.1C; 315.1C	5	B4
LPAZ092	16181G; 16189C; 16217C	73G; 185A; 249d; 263G; 290d; 291d; 309.1C; 315.1C[a]	1	H
LPAZ093	16209C; 16223T; 16311C	73G; 150T; 152C; 189G; 195C; 200G; 263G; 309.1C; 315.1C[a]	1	M7a
LPAZ094	16182C; 16183C; 16189C; 16223T; 16298C; 16325C; 16327T; 16344T[a]	73G; 263G; 309.1C; 309.2C; 315.1C[a]	1	M8/N9b
LPAZ095	16183C; 16188T; 16189C; 16217C	263G; 309.1C; 315.1C[a]	2	B4
LPAZ096	16140C; 16183C; 16188T; 16189C; 16217C	263G; 315.1C	2	B4
LPAZ097	16183C; 16188T; 16189C; 16217C	263G; 309.1C; 309.2C; 315.1C[a]	4	B4

[a] Means length heteroplasmy.

ascertain the identity of victims of mass disasters or terrorism, it is important to have a high quality population database at disposal that allows to estimate the frequency of a questioned haplotype.[7] At the moment, however, mitochondrial DNA databases are limited in representative population data of South American origin. This paper presents a contribution to the worldwide collection of mtDNA sequences set on HVI and HVII haplotypes from La Paz for forensic and phylogenetic purposes.

The multiple mtDNA copies in one individual are in general identical, a condition known as homoplasmy. But homoplasmy is not universal and the co-existence of different mtDNA sequences in one individual is possible, a condition identified as heteroplasmy. Heteroplasmy is more frequently detected in the control region as length variants, named length heteroplasmy. Point heteroplasmy is the product of a nucleotide change at a certain position that some sequences have and others do not. It has been determined that, at present, the rate of heteroplasmy in one population is 14%.[8]

This paper reports the mtDNA of one population from La Paz (Bolivia) composed of haplotypes from 110 native individuals, the distribution of the haplogroups in the population and a phylogenetic study in relation to other populations.

The aims of this work were to (i) start the creation of a mtDNA database of the La Paz population, (ii) obtain the mtDNA variability, (iii) classify the haplotypes into haplogroups in order to discover the genetic structure of La Paz population through mtDNA and (iv) infer the evolutionary processes that have formed the history of the La Paz population.

2. Material and methods

Blood samples were obtained respecting the informed consent established in Bolivia from 110 healthy and unrelated individuals with ages between sixteen and seventy two years old. All of the individuals are from the La Paz department of Bolivia. DNA was extracted using the Chelex®100 method.[9] The hypervariable regions of the mtDNA non-coding control region were amplified using the primers L15997 and H16401 for HVI and the primers L408 and H048 for HVII[10] and thermocycling conditions were performed in a GeneAmp® PCR system 2700 (Applied Biosystems, Foster City, CA). PCR amplification was carried out using 1X Buffer PE II, $MgCl_2$ at 2 mM; dNTPs at 0.2 mM each, primers at 0.2 μM, 5 U/μl of AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA) and 2 ng of genomic DNA in a 25 μL final reaction volume. Thermocycling conditions was as follows: at 95 °C for 6 min, followed by 32 cycles of 10 s at 95 °C, 30 s at 60 °C, 30 s at 72 °C, and finally at 15 °C for 1 min. Both light and heavy chains of each hypervariable region were sequenced using the sequencing chemistry ABI Prism® dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). The sequencing cycle was performed in a GeneAmp® PCR system 2700 (Applied Biosystems, Foster City, CA) in a 25 cycles: at 96 °C for 10 s, 56 °C for 5 s, and 72 °C for 4 min. The nucleotide sequence of HVI from position 16024 until position 16390, and nucleotide sequence of HVII from position 73 until position 340 were analysed in the ABI PRISM® sequencers 3100 – Avant Genetic Analyser (with the DNA Sequencing Analysis

Table 2
Diversity parameters for mtDNA sequences of 110 individuals from La Paz.

MtDNA control region	Nucleotide diversity (μ)	Sequence diversity (h)
HV1	0.011	0.948
HV2	0.015	0.986
HV1 + HV2	0.015 ± 0.0075	0.996

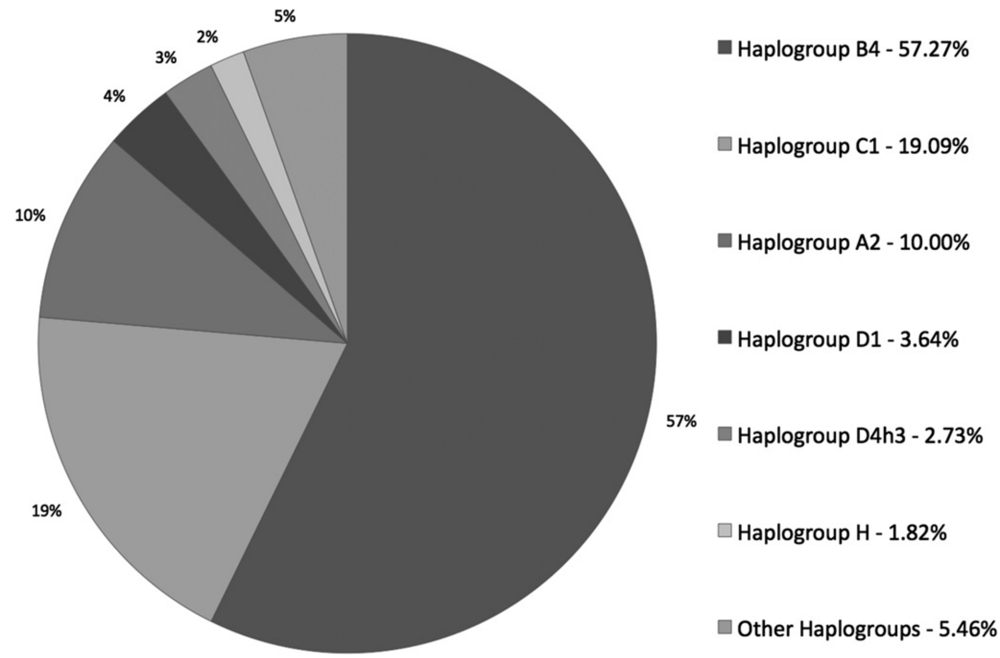

Fig. 1. Haplogroup distribution of mtDNA in La Paz, Bolivia.

Software™ v.3.7 and the SeqScape® Software v.2.0) and in the 3130 – Genetic Analyser (with the ABI DNA Sequencing Analysis Software v.5.2 and the SeqScape® Software v.2.5). The obtained haplotypes were compared with the Cambridge Reference Sequence (CRS)[11,12] and the differences found in each sequence regarding the CRS were typed following the nomenclature recommendations of the IUPAC.[13] The establishment of the haplogroups was made considering the nucleotide substitutions on non-coding hypervariable regions I and II (HVI + HVII) on a Web-based tool for management and quality analysis of mitochondrial DNA[14] based on the specific control region mutation motifs.

Statistical analysis for HVI and HVII regions was made separately, calculating the nucleotide and sequence diversity[15] and the same parameters were also calculated for both regions[16] using the Arlequin v.3.0 software.[17] The phylogenetic study was made by analysing the molecular distances between the studied population and chosen populations from the five continents.[18–33] Genetic affinities among populations were computed using Arlequin v.3.0 software (Excoffier et al., 2005). The phylogenetic representation obtained from the genetic distances was made with the Neighbour method of Phylip v.3.5C software[34] and the tree was obtained with Treeview 1.5.2. software.[35]

3. Results and discussion

In this study, 97 haplotypes were identified, 90 of them being unique, expressed by 122 polymorphic nucleotide positions (Table 1). The analysing HVI and HVII regions separately, 67 different haplotypes were recognized for the HVI region (55 unique) and 71 different haplotypes for the HVII region (52 unique). The highest density of polymorphic sites was obtained for HV1, which contains 69 polymorphic sites in total length 367 bp. HV2 region presents 53 polymorphic sites in 268 bp. The most common polymorphism detected in the HV1 region was the single nucleotide substitution T16189C, which is present in 65.5% of the sequences, followed by the T16217C, polymorphism that appeared in 64.5% of the samples. Also common in this Bolivian sequences is the polymorphism in position 16183 (62.7%) that appear as a transversion A16183C (60.9%), or as a transition A16183G (0.9%), or deleted (0.9%). In HV2 region, beyond the polymorphic site A263G and 315.1C that appear in all the human sequences, the single nucleotide variant A73G has a very high rate of substitution in the sequences (91.8%). At only 3 out of 122 positions (2.46%) more than one mutation events were noticed, all in the HV1 region. A 16105K (T/G) point heteroplasmy was found in HVI region in sequence LPAZ038 and a 162Y (T/C) point heteroplasmy in the HVII region in sequence

Table 3
"Estimated haplogroup" and "expected haplogroup" designed by mtDNAmanager for sequences LPAZ015, LPAZ017, LPAZ027, LPAZ038, LPAZ041, LPAZ092 and LPAZ096 based on an individual query. N – number of occurrence of the haplotype; Est. Hg – "estimated haplogroup"; Exp. Hg – "expected haplogroup".

	HVI	HVII	N	Est. Hg	Exp. Hg
LPAZ015	16168T; 16183C; 16189C; 16217C; 16270T; 16278T[a]	73G; 263G; 315.1C	1	B4	B4f
LPAZ017	16168T; 16182C; 16183C; 16189C; 16217C; 16218T[a]	73G; 263G; 315.1C	1	B4	B4f
LPAZ027	16183C; 16189C; 16217C; 16261T; 16319A[a]	73G; 75A; 263G; 315.1C; 340T[a]	1	B4	B4a
LPAZ038	16105T/G; 16168T; 16183C; 16189C; 16217C; 16301A[a]	73G; 204C; 263G; 309.1C; 315.1C[a]	1	B4	B4f
LPAZ041	16066G; 16183d; 16186T; 16189C; 16217C	73G; 143A; 210G; 263G; 309.1C; 315.1C	1	H	B4
LPAZ092	16181G; 16189C; 16217C	73G; 185A; 249d; 263G; 290d; 291d; 309.1C; 315.1C[a]	1	H	B4
LPAZ096	16140C; 16183C; 16188T; 16189C; 16217C	263G; 315.1C	2	B5	B4c1b

[a] Means length heteroplasmy.

Fig. 2. Phylogenetic tree: genetic distances between populations from the five continents.

LPAZ061 (Table 1).[36] Populations with a high frequency of unique haplotypes are consistent with an early local settlement.[5]

For this population the values of sequence diversity are 0.948 and 0.986 for HVI and HVII regions, respectively. The sequence diversity when both hypervariable regions are analysed together is 0.996. The nucleotide diversity for HVI region is 0.011 and for HVII region is 0.015. The nucleotide diversity for both sequenced regions is 0.015 ± 0.0075 (Table 2). The diversity parameters were higher for the HVII region than for the HVI region and sequence diversity increases significantly when both regions are analysed together, which means that the analysis of the HVII region adds value to the population study. The obtained diversity parameters of the non-coding hypervariable region I (HVI) are similar to the ones described in the literature on Native American populations.[37]

The rate of length heteroplasmy is 36.36% in HVI region and 52.73% in HVII region. It should be noticed that the high rate of length heteroplasmy that was found, which is much higher than in Caucasian populations, is similar to other populations from Southwest Asia, namely the Japanese and Korean populations. The most frequent polymorphisms found in this study are also frequent in the Korean population.[38,39]

It was possible to include 100% of the mtDNA sequences into a specific mtDNA haplogroup according to nucleotide substitutions of the HVI and HVII regions. Since the mtDNAmanager is a dynamic Web-based tool that follows the evolution of the state of the art, the presented results refer to the analysis performed on 8th June 2009. The bioinformatics tools of mtDNAmanager designate the "estimated haplogroup" and the "expected haplogroup".[14] On Table 1, it is presented the "estimated haplogroup" that is designated when the query data indicate the presence of accompanying mutations additional to the clear diagnostic mutations. The "expected haplogroup" is elected when the query data possess clear diagnostic mutations. The haplogroup distribution (Fig. 1) is as follows: 57.27% B4, 19.09% C1, 10.00% A2, 3.64% D1, 2.73% D4h3, 1.82% H, and 0.91% for each of the haplogroups A4, B4c1a, CZ, D4J, M7a and M8/N9b. The majority of lineages, 96.36%, have polymorphisms belonging to major haplogroups A, B, C or D, characteristic of South American populations[40–42] and 3.64% of the lineages belong to West Eurasian Haplogroup H and East Asian haplogroups M7a and M8/N9b. Events related to colonial activities, modern migration or recent admixture between co-existing Native Americans and European populations can explain the appearance of the haplogroup H, which is a minority in the study. For sequences LPAZ041 and LPAZ092 (Table 3) the "estimated haplogroup" on mtDNAmanager was haplogroup H, however these haplotypes have the specific motif for haplogroup B4 (16217C) and the "expected haplogroup" in mtDNAmanager for these sequences is haplogroup B4. Also, for sequences LPAZ015, LPAZ017, LPAZ027, LPAZ038 and LPAZ096 mtDNAmanager designated a different "estimated haplogroup" and an "expected haplogroup", so we made an individual query for each of these sequences (Table 3). The present haplogroup results are in concurrence with previous studies based on the mtDNA analysis of American populations.[23,37,43–46] A study implemented in native Bolivian populations showed that the haplogroup B has a higher frequency in the highlands and the haplogroup C is more representative in the lowlands. In the Aymara group the haplogroup B was the most frequent and in Quichua group the haplogroups C and A were more representative.[43]

By the analysis of the phylogenetic tree (Fig. 2) obtained from the molecular distances of populations, it was found that the Bolivian population reveals lower genetic distance to the Korean ($\Phi st = 0.09976$, $P = 0.00000$), Chilean aboriginal Pehuenche ($\Phi st = 0.10402$, $P = 0.00030$), Japan ($\Phi st = 0.11130$, $P = 0.00000$) Chilean aboriginal Mapuche ($\Phi st = 0.13124$, $P = 0.00000$) and Australian ($\Phi st = 0.14335$, $P = 0.00000$) populations. Nevertheless, genetic distance analysis resulted in a high significant differentiation between Bolivian population and any other population of the comparative study.

Recent studies based on mtDNA presented evidences for a "Three-stage model" consisting on an early expansion into Beringia followed by ~20,000 years of population stability before the final entry into the Americas.[47] But in spite of this theory, a maritime entrance in South America[48] through the Pacific Ocean has been marking position as a result of archaeological findings along the different areas of South America. On a recent work, Dillehay et al., 2008[49] found on archaeological site Monte Verde in Southern Chile remains of nine species of marine algae dated between 14,220 and 13,980 calendar years before present. Our phylogenetic results – namely the lower genetic distance to Korean – make us believe that the settlement of South America was also along the Pacific coast.

4. Conclusions

Due to the high frequency of unique haplotypes, the studied population shows a great genetic variability. The nucleotide and sequence diversity are high and similar to other populations of South America. The diversity parameters are higher for HVII region than for HVI region and when both regions are analysed together the diversity parameters increase. Native Bolivians from La Paz exhibited haplotypes that belonged to the haplogroups observed in Native Americans and the most represented is haplogroup B4. Phylogenetic analysis reveals proximity to the Korean, Chilean aboriginal, Japanese and Australian populations, but the Bolivian population presents significant differences when compared with all of the other chosen populations. Interpopulation genetic variability was high, suggesting an early settlement of the population.

Conflicts of interest

None declared.

References

1. Klein H. *A concise history of Bolivia*. Cambridge: Cambridge University Press; 2003.
2. Giles RE, Blanc H, Cann HM, Wallace DC. Maternal inheritance of human mitochondrial DNA. *Proc Natl Acad Sci USA* 1980;**77**(11):6715–9.
3. Ballard JW, Whitlock MO. The incomplete natural history of mitochondria. *Mol Ecol* 2004;**13**(4):729–44.
4. Van Oven M, Kayser M. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Hum Mutat* 2008;**29**:E386–E394.
5. Torroni A, Schurr TG, Cabell MF, Brown MD, Neel JV, Larsen M, et al. Asian affinities and continental radiation of the four founding Native American mtDNAs. *Am J Hum Genet* 1993;**53**(3):563–90.
6. Brown MD, Hosseini SH, Torroni A, Bandelt HJ, Allen JC, Schurr TG, et al. MtDNA haplogroup X: an ancient link between Europe/Western Asia and North America? *Am J Hum Genet* 1998;**63**(6):1852–61.
7. Schneider PM. Scientific standards for studies in forensic genetics. *Forensic Sci Int* 2007;**165**:238–43.
8. Tully LA, Parson TJ, Steighner RJ, Holland MM, Marino MA, Prenger VL. A sensitive denaturing gradient-gel electrophoresis assay reveals a high frequency of heteroplasmy in hypervariable region 1 of the human mtDNA control region. *Am J Hum Genet* 2000;**67**(2):432–43.
9. Walsh PS, Metzger DA, Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 1991;**10**(4):506–13.
10. Wilson MR, DiZinno JA, Polanksey D, Replogle J, Budowle B. Validation of mitochondrial DNA sequencing for forensic casework analysis. *Int J Legal Med* 1995;**108**:68–74.
11. Anderson S, Bankier AT, Barrel BG, De Bruijn MH, Coulson AR, Drouin J, et al. Sequence and organization of the human mitochondrial genome. *Nature* 1981;**290**:457–65.
12. Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat Genet* 1999;**23**(2):147.
13. Carracedo A, Bär W, Lincoln P, Mayr W, Morling N, Olaisen B, et al. DNA Commission of the international society for forensic genetics: guidelines for mitochondrial DNA typing. *Forensic Sci Int* 2000;**110**(2):79–85.

14. Lee HY, Song I, Ha E, Cho S-B, Yang WI, Shin K-J. mtDNAmanager: a web-based tool for the management and quality analysis of mitochondrial DNA control-region sequences. *BMC Bioinformatics* 2008;**9**:483.
15. Nei M, Tajima F. DNA polymorphism detectable by restriction endonucleases. *Genetics* 1981;**97**(1):145–63.
16. Nei M. *Molecular evolutionary genetics*. New York: Columbia University Press; 1987.
17. Excoffier L, Laval G, Schneider S. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evol Bioinform Online* 2005;**1**:47–50.
18. Piercy R, Sullivan KM, Benson N, Gill P. The application of mitochondrial DNA typing to the study of white Caucasian genetic identification. *Int J Legal Med* 1993;**106**(2):85–90.
19. Lutz S, Weisser H-J, Heizmann J, Pollak S. Location and frequency of polymorphic positions in the mtDNA control region of individuals from Germany. *Int J Legal Med* 1998;**111**(2):67–77.
20. Parson W, Parsons TJ, Scheithauer R, Holland MM. Population data for 101 Austrian Caucasian mitochondrial DNA d-loop sequences: application of mtDNA sequence analysis to a forensic case. *Int J Legal Med* 1998; **111**(3):124–32.
21. Rousselet F, Mangin P. Mitochondrial DNA polymorphisms: a study of 50 French Caucasian individuals and application to forensic casework. *Int J Legal Med* 1998;**111**(6):292–8.
22. Crespillo M, Luque JA, Paredes M, Fernández R, Ramírez E, Valverde JL. Mitochondrial DNA sequences for 118 individuals from northeastern Spain. *Int J Legal Med* 2000;**114**(1–2):130–2.
23. Moraga ML, Rocco P, Miquel JJ, Nervi F, Llop E, Chakraborty R, et al. Mitochondrial DNA polymorphisms in Chilean aboriginal populations: implications for the peopling of the southern cone of the continent. *Am J Phys Anthropol* 2000;**113**(1):19–29.
24. Pereira L, Macaulay V, Torroni A, Scozzari R, Prata MJ, Amorim A. Prehistoric and historic traces in the mtDNA of Mozambique: insights into the Bantu expansions and the slave trade. *Ann Hum Genet* 2001;**65**(Pt5):439–58.
25. Budowle B, Allard MW, Fisher CL, Isenberg AR, Monson KL, Stewart JE, et al. HVI and HVII mitochondrial DNA in Apaches and Navajos. *Int J Legal Med* 2002;**116**(4):212–5.
26. Carvalho M, Antunes H, Anjos MJ, Andrade L, Lopes V, Corte-Real F, et al. *Estudo da Variabilidade do DNA Mitocondrial em Populações Africanas (Guiné e Cabo Verde)*. Barcelona, Spain: VII Jornadas de Genética Forense, Spanish and Portuguese Group of ISFG; 2002.
27. Carvalho M, Mendes C, Antunes H, Anjos MJ, Andrade L, Lopes V, et al. mtDNA analysis in Portuguese populations (Central Portugal and Azores Islands): polymorphic sites in control region sequences. In: Brinkmann B, Carracedo A, editors. *Progress in forensic genetics*, vol. 9. Elsevier Science B.V; 2003. p. 535–9.
28. Trovoada MJ, Pereira L, Gusmão L, Abade A, Amorim A, Prata MJ. Pattern of mtDNA variation in three populations from Sao Tome e Príncipe. *Ann Hum Genet* 2004;**68**:40–54.
29. Vona G, Falchi A, Moral P, Calò CM, Varesi L. Mitochondrial sequence variation in the Guahibo Amerindian population from Venezuela. *Am J Phys Anthropol* 2005;**127**:361–9.
30. Carvalho M, Balsa F, Lopes V, Anjos MJ, Andrade L, Serra A, et al. *Mitochondrial DNA analysis in a population from Angola*. Budapest, Hungary: XX™ Congress of International Academy of Legal Medicine; 2006.
31. Ingman M, Gullensten U. mtDB: human mitochondrial genome database, a resource for population genetics and medical sciences. *Nucleic Acids Res* 2006;**34**:D749–D751.
32. Shin M-G, Levin BC, Kim H-R, Lee I-K, Cho D, Kee SJ, et al. Profiling of length heteroplasmies in the human mitochondrial DNA control regions from blood cells in the Korean population. *Electrophoresis* 2006;**27**:1331–40.
33. Torres MM, Bravi CM, Bortolini M-C, Duque C, Callergari-Jacques S, Ortiz D, et al. A revertant of the major founder Native American haplogroup C common in populations from northern South America. *Am J Hum Biol* 2006;**18**(1):59–65.
34. Felsenstein J. PHYLIP: phylogeny interface package (version 3.2). *Cladistics* 1989;**5**:164–6.
35. Page RDM. Treeview: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* 1996;**12**:357–8.
36. Nomenclature Committee of the International Union of Biochemistry, NC-IUB, http://www.chem.qmul.ac.uk/iubmb/misc/naseq.html.
37. Lewis CM, Tito RY, Lizárraga B, Stone AC. Land, language, and loci: mtDNA in Native Americans and the genetic history of Peru. *Am J Phys Anthropol* 2005;**127**(3):351–60.
38. Imaizumi K, Parsons TJ, Yoshino M, Holland MM. A new database of mitochondrial DNA hypervariable regions I and II sequences from 162 Japanese individuals. *Int J Legal Med* 2002;**116**(2):68–73.
39. Jin HJ, Kwark KD, Hong SB, Shin DJ, Han MS, Tyler-Smith C, et al. Forensic genetic analysis of mitochondrial DNA hypervariable region I/II sequences: an expanded Korean population database. *Forensic Sci Int* 2006;**158**(2–3):125–30.
40. Forster P, Harding R, Torroni A, Bandelt H-J. Origin and evolution of Native American mtDNA variation: a reappraisal. *Am J Hum Genet* 1996;**59**:935–45.
41. Tamm E, Kivisild T, Reidla M, Metspalu M, Smith GD, Mulligan CJ, et al. Beringian standstill and soared of Native American founders. *PLoS One* 2007;**2**(9):e829.
42. Achilli A, Perego UA, Bravi CM, Coble MD, Kong Q-P, Woodward SR, et al. The phylogeny of the four Pan-American MtDNA haplogroups: implication for evolutionary and disease studies. *PLoS ONE* 2008;**3**(3):e1764.
43. Bert F, Corella A, Gené M, Pérez-Peréz A, Turbón D. Major mitochondrial DNA haplotype and heterogeneity in highland and lowland Amerindian populations from Bolivia. *Hum Biol* 2001;**73**(1):1–16.
44. Malhi RS, Mortensen HM, Eshleman JA, Kemp BM, Lorenz JG, Kaestle FA, et al. Native American mtDNA prehistory in the American Southwest. *Am J Phys Anthropol* 2003;**120**(2):108–24.
45. García F, Moraga M, Vera S, Henríquez H, Llop E, Aspillaga E, et al. mtDNA microevolution in Southern Chile's archipelagos. *Am J Phys Anthropol* 2006;**129**(3):473–81.
46. Sans M, Merriwether DA, Hidalgo PC, Bentancor N, Weimer TA, Franco MH, et al. Population structure and admixture in Cerro Largo, Uruguay, based on blood markers and mitochondrial DNA polymorphisms. *Am J Hum Biol* 2006;**18**(4):513–24.
47. Fagundes N, Kanitz R, Bonatto S. A reevaluation of the Native American mtDNA genome diversity and its bearing on the models of early colonization of Beringia. *PLoS One* 2008;**3**(9):e3157.
48. Fix AG. Rapid deployment of the five founding Amerind mtDNA haplogroups via coastal and reverine colonization. *Am J Phys Anthropol* 2005;**128**(2):430–6.
49. Dillehay TD, Ramírez M, Pino M, Collins MB, Rossen J, Pino-Navarro JD. Monte Verde: seaweed, food, medicine, and the peopling of South America. *Science* 2008;**320**(5877):784–6.